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The Adsorption of Suspended MH129F Cells to DEAE-Sephadex Particles

Shoshi INOOKA

Department of Animal Husbandry, Facutly of Agriculture
Tohoku University, Sendai, Japan
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Summary

This experiment was carried out using DEAE-sephadex as an ion exchanger, to clarify whether or not the adsorption of cells on the DEAE-sephadex will occur if it is mixed into the cell suspensions. The results presented in this paper are as follows:

1) The cells adsorbed on the surface of each DEAE-sephadex particle are observed within one minute after the particles in 0.15 M NaCl solution were added to the cells suspended in 0.15 M NaCl solution;

2) The phenomenon is also observed in the case of the use of DEAE-cellulose instead of DEAE-sephadex, but no adsorption was found on the Sephadex particles;

3) The separation of the cells from the DEAE-sephadex is observed when the cells adsorbed were brought into 0.30, 0.40, 0.50 and 0.60 M NaCl solution. Moreover, the adsorption is observed to recur when the separated cells were again replaced in 0.15 M NaCl solution.

From the results obtained, the possibility of employing DEAE-sephadex as a means of investigation in cell physiology was discussed.

It is well known that ion exchangers have been widely used to isolate and purify a substance. But it remains unknown whether the ion exchangers can be used to an investigation of cytology. This experiment was carried out, using DEAE-sephadex as an ion exchanger, to clarify whether or not the adsorption of cells on the DEAE-sephadex will occur if it is mixed into cell suspensions. From the results obtained, whether ion exhangers can be employed as a means of investigation in cytology is discussed in this paper.

Experimantal Procedures and Results

MH129F cell suspensions.

The MH129F cells (1) obtained from DD mice transplanted into the ascitic form were used in this experiment. The cells were washed twice with buffered

saline and 0.15 M NaCl solution, respectively. The concentration was adjusted to 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 per 0.2 ml of 0.15 M NaCl solution.

DEAE-sephadex particles.

0.5 g of DEAE-sephadex (A–50, capacity, 3.5±0.5 meg/g, particle size, 40–120μ, Pharmacia Uppsala, Sweden) (Fig. 1) was suspended with the use of 0.15 M NaCl solution, pH 6.2. The concentration was adjusted to 10^3, 10^4 and 10^5 per 0.3 ml of 0.15 M NaCl solution. DEAE-cellulose (Serva) and Sephadex (G–200, water regain, 20±2 g/s, particle size, 40–120μ, Pharmacia Uppsala, Sweden) were prepared in the usual manner.

The adsorption of the MH129F cells to DEAE-sephadex particles.

Each suspension of the MH129F cells adjusted to contain 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 cells in 0.2 ml was put in test tubes. Each suspension of the particles adjusted to contain 10^3, 10^4 and 10^5 of DEAE-sephadex particles in 0.3 ml was added to MH129F cells of the various concentrations. Then, the mixture of the MH129F cells and DEAE-sephadex particles were shaken by hand at room temperature. After1/4, 1/2, 1, 2 and 3 minutes of shaking, one drop of each mixture was immediately placed on the slide glass, and it was examined under a magnification of 150 X with a binocular microscope. Also, the adsorption to the MH129F cells of DEAE-cellulose or Sephadex particles was observed.

The cells adsorbed on the surface of Sephadex particles were detected when the DEAE-sephadex particles were added to the cell suspensions. In the case of the mixture with the rate of 100 cells per particle, the great majority of the MH129F

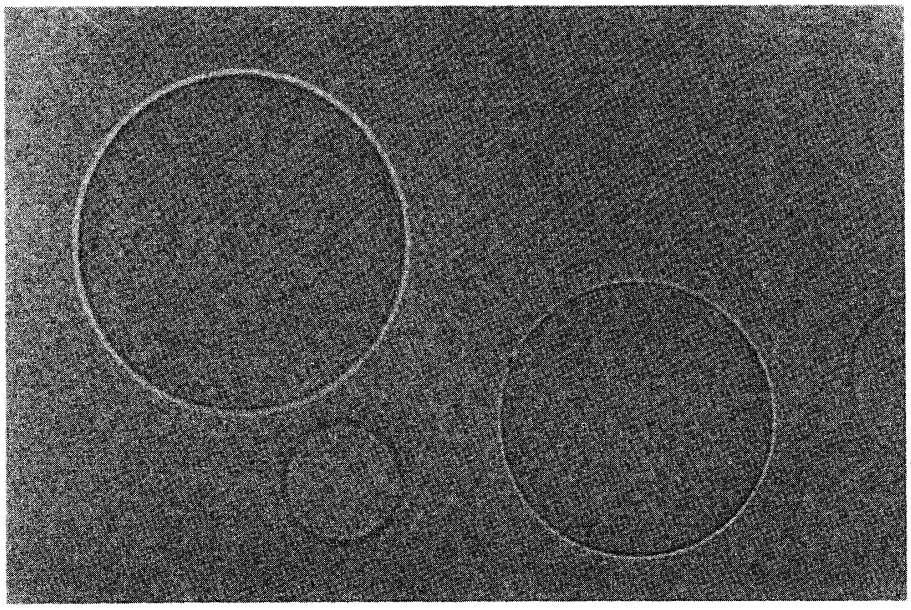

FIG. 1. The DEAE sephadex particles before the mixture with the MH129F cells.

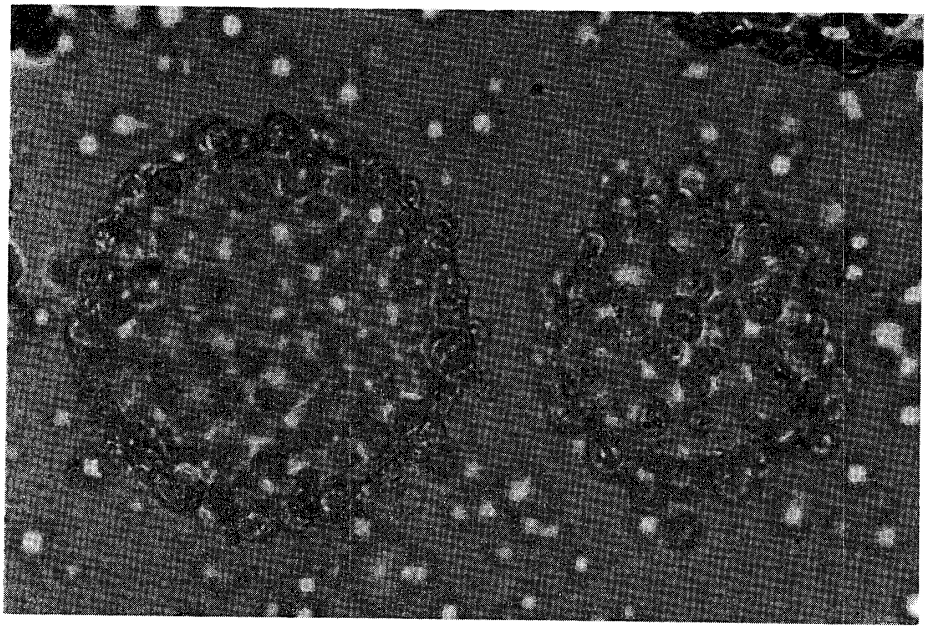

FIG. 2. The DEAE-sephadex particles after the mixture with the MH129F cells. The adsorption of the cells over 50 per a particle is ovserved.

cells adsorbed on the surface of each DEAE-sephadex particle were observed. They were too numerous to count the exact number of cells when over 50 per each DEAE-sephadex particle were observed (Fig. 2). Also, the number of cell adsorbed was over the range 10 to 50 at the rate of 10 cells per particle and under 10 at the rate of a cell per particle (Table 1).

This adsorption was observed within 1/4 minutes after the mixture and became quite marked by 1/2 to 1 minute (Fig.5).

On the other hand, in the mixture with Sephadex-particles, little or no adsorption of the MH129F cells on the particles occurred, even with one minute shaking (Fig. 3).

However, the cells adsorbed on a piece of cellulose were detected when the cells were mixed with DEAE-cellulose instead of DEAE-sephadex (Fig. 4).

TABLE I. *The Adsorption of the Cells to the DEAE-sephadex Particles in Mixtures of Various Rates of Cell to the Particles.* The Number of the Cells Adsorbed on 100 Particles: — 0, + 1–9, ++ 10–50, +++ over 50

		The concentrations of the cells.					
		10^2	10^3	10^4	10^5	10^6	10^7
The concentrations of the particles.	10^5	—	—	—	+	++	+++
	10^4	—	—	+	++	+++	+++
	10^3	—	+	++	+++	+++	+++

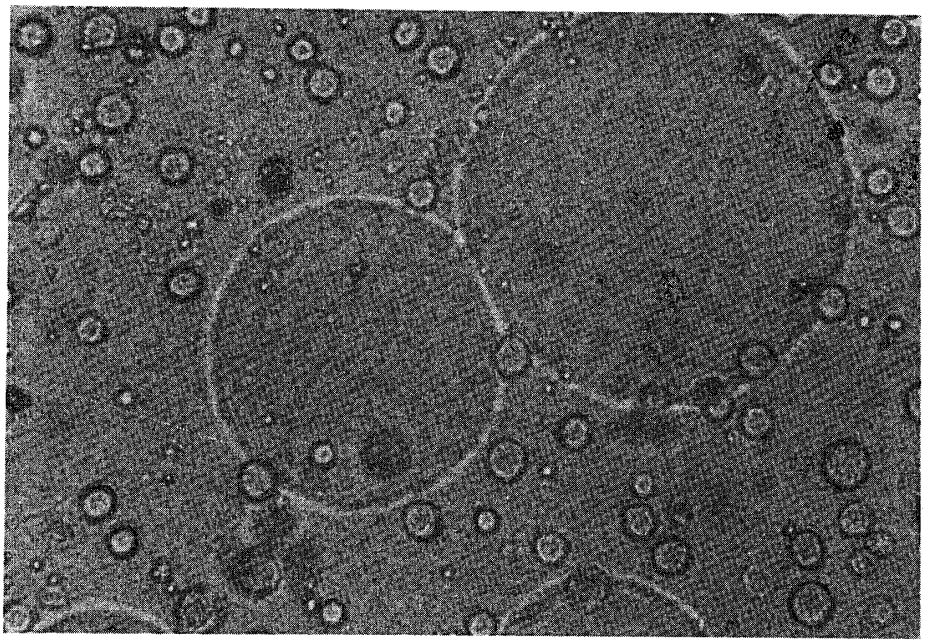

FIG. 3. The sephadex particles in mixture with the MH129F cells. No adsorption is observed.

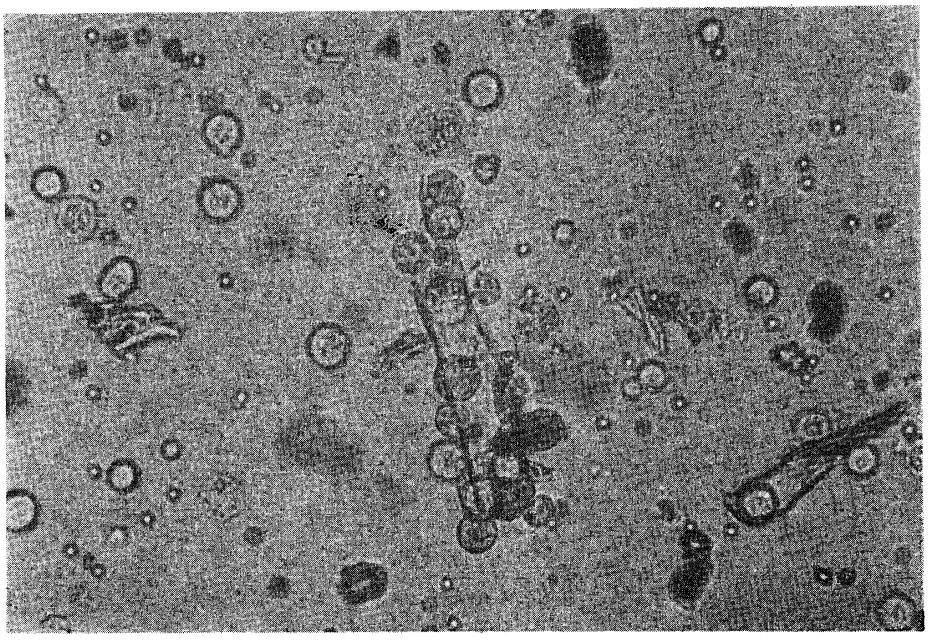

FIG. 4. The DEAE-cellulose in mixture with the MH129F cells. Cells adsorbed on a peace of the cellulose are observed.

The separation of the MH129F cells from DEAE-sephadex particles on which the cells were adsorbed.

The concentration of MH129F cells was adjusted to 10^7 per 0.2 ml. On the other hand, the concentration of DEAE-sephadex particles was adjusted to 10^5

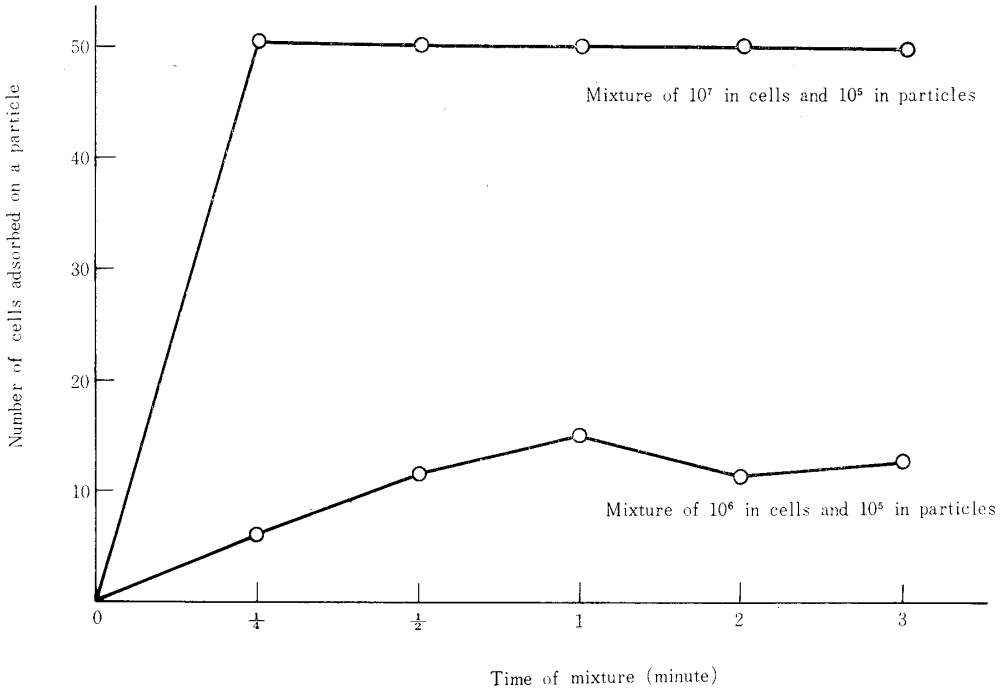

FIG. 5. The number of cells adsorbed on a DEAE-sephadex particle after mixture of cells and the particles.

per 0.3 ml. One ml of the cells and 1.5 ml of the particles were mixed in the test tube. The mixture was shaken by hand for one minute at room temperature. The contents were divided into 5 tubes. Then, about 5 ml of NaCl solution of each concentration (0.15, 0.30, 0.40, 0.50 and 0.60 M) were added to each tube containing the mixture with particles and cells. The mixtures were washed with these solutions. After washing again, 0.5 ml of each NaCl solution was added to each cell and particle residue. Each mixture cell and particle residue was suspended with pipetting. One drop of each suspension was placed on the slide glass. The number of cells adsorbed on 100 of DEAE-sephadex particles was counted. On the other hand, the reminder of each tube was sedimented by centrifugation. The sedimented cells and particles were washed twice with about 5 ml of 0.15 NaCl solution. After washing, 0.5 ml of 0.15 M NaCl solution was added to each cell and particle residue. Then, one drop of each suspension was placed on a glass slide. The number of the cells adsorbed on 100 of the DEAE-sephadex particles was counted.

As mentioned above, the adsorption of the cells over 50 per each DEAE-sephadex particle was detected when the cells and the particles were mixed in 0.15 M NaCl solution (Fig. 6). When the cells adsorbed were brought into 0.30, 0.40, 0.50 and 0.60 M NaCl solution, separation of the cells from the DEAE-sephadex particles on which the cells were adsorbed was observed. The rate of the separation was 75 per cent in 0.30 M NaCl solution, as shown in Fig. 8.

Most of the cells were observed to be separated from the particles in 0.60 M NaCl solution (Fig. 7).

Also, readsorption of the cells to the particles was observed when the cells which were separated in 0.30, 0.40, 0.50 and 0.60 M NaCl solution were again

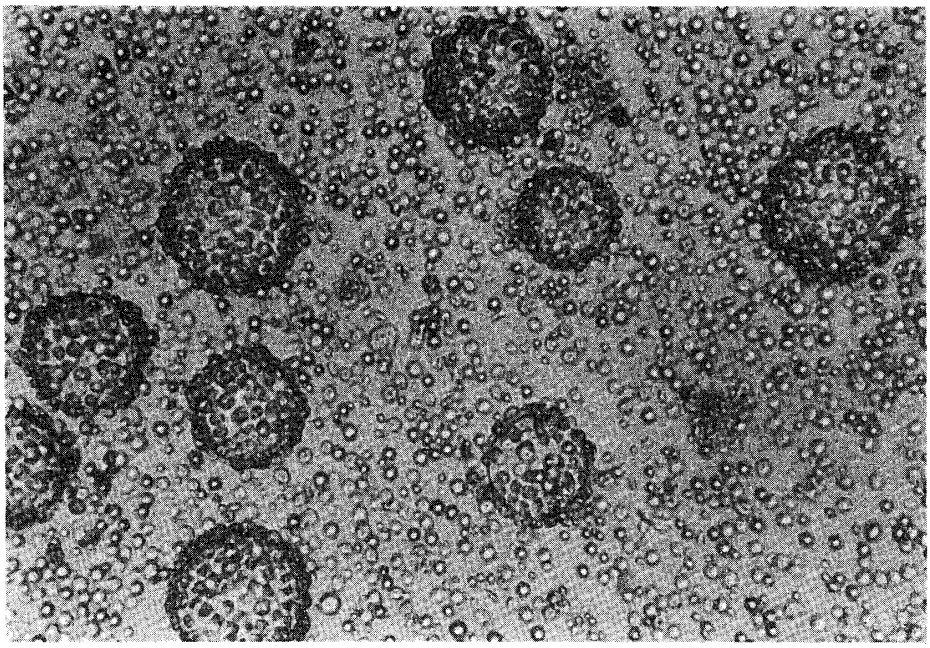

FIG. 6. The mixture of DEAE-sephadex particles and MH129F cells at the rate of loo cells per particle. The adsorption of over 50 cells per particle is observed.

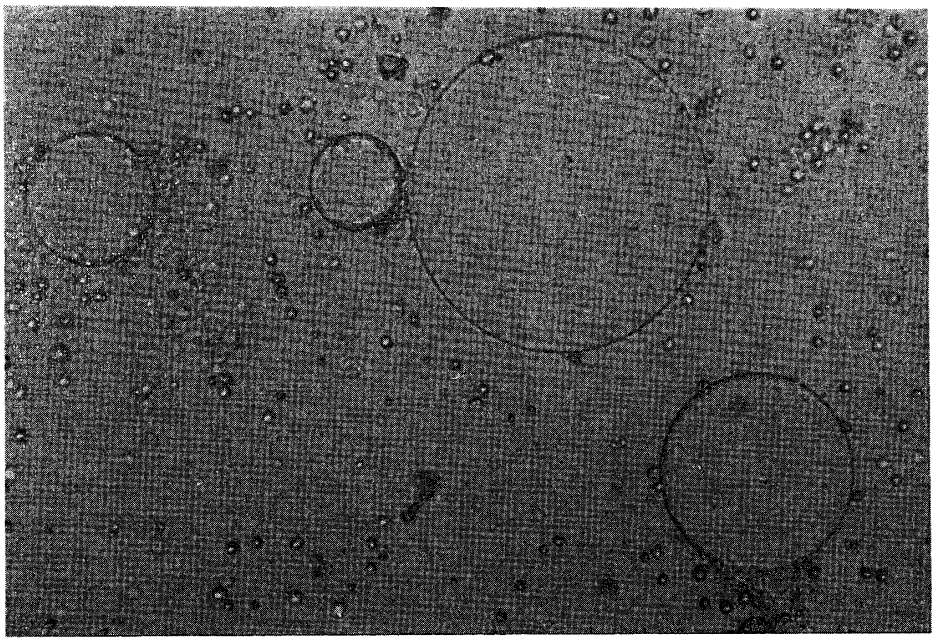

FIG. 7. The DEAE-sephadex particles when the cells adsorbed in FIG. 6 were brought into 0.60 M NaCl solution. Little or no adsorption of the cells on each particle is observed.

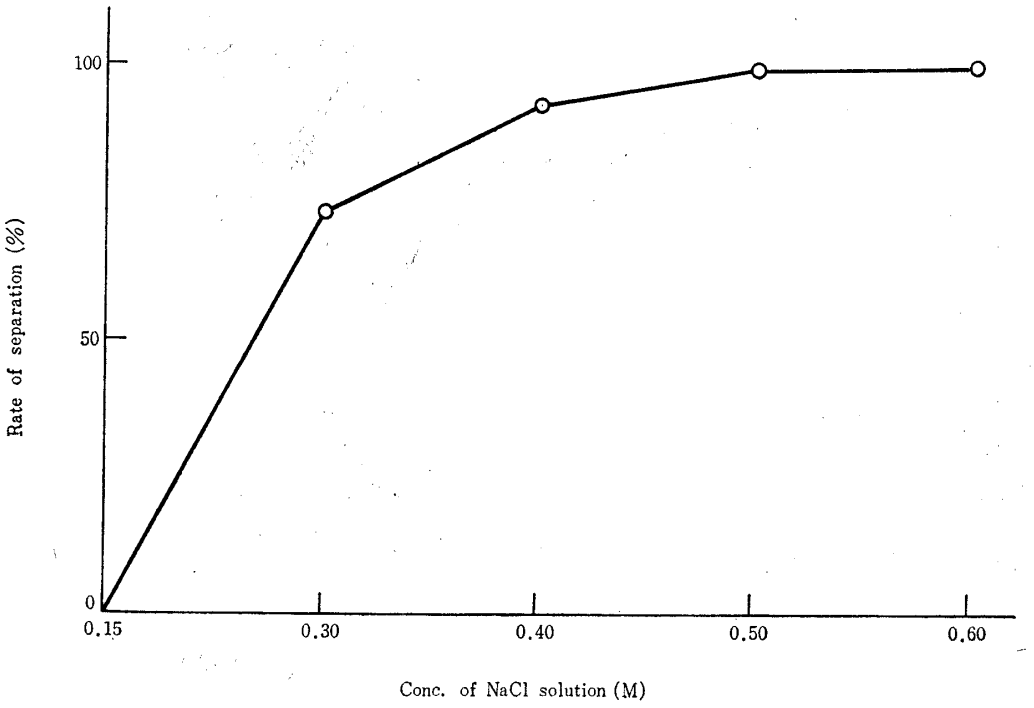

FIG. 8. The separation of the cells from the DEAE-sephadex particles.

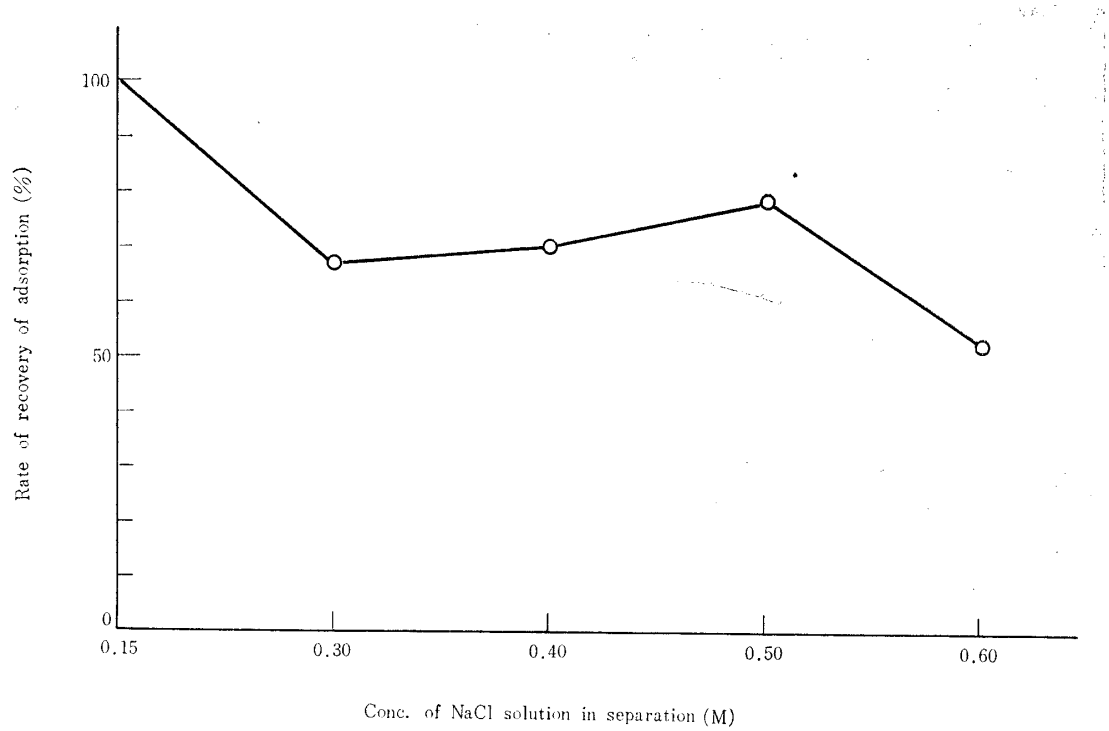

FIG. 9. The readsorption of the cells which were separated from the DEAE-sephadex parlticles.

placed in 0.15 M NaCl solution. The rate of the recovery of the adsorption was 70 per cent in the cells obtained from 0.30 M solution, 50 per cent in the cells of 0.60 M solution (Fig. 9).

Comment

The adsorption of MH129F cells to DEAE-sephadex particles was observed in the mixture with the cells and the particles. This phenomenon, also, was observed in the use of DEAE-cellulose. However, in the case of the mixture with Sephadex particles, no adsorption was observed. This fact indicates that the adsorption of the cells to DEAE-sephadex particles depends on the DEAE in DEAE-sephadex particle.

On the other hand, it is well known that a cell membrane generally has a negative charge. From this view point, it was suggested that this phenomenon was caused by the ion binding between some ion groups of the cell membrane or cell surface and DEAE-sephadex. Moreover, it was noted that the separation or readsorption between the cells and the DEAE-sephadex particles was observed to be according to the concentration of the NaCl solution. Thus, this method may be usable to isolate a cell population which has the same charge. Namely, DEAE-sephadex may be employed in some important aspects in cytology: the detection or separation of a cell population from various cell populations and a cell from various kinds of cells.

References

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